

# Rainfastness of a Microencapsulated Sex Pheromone Formulation for Codling Moth (Lepidoptera: Tortricidae)

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**ABSTRACT** The rainfastness of a microencapsulated sex pheromone formulation for codling moth, *Cydia pomonella* (L.), was evaluated in a series of laboratory experiments with detached apple, pear, and walnut leaves. Increasing the intensity and duration of simulated rainfall significantly increased the removal of microcapsules from both the top and bottom of apple leaves. The removal of microcapsules was significantly higher from the top versus the bottom of leaves at all rates tested. Leaf angle was a significant factor affecting the removal of microcapsules from the top surface of apple leaves with fewer microcapsules removed, because leaves were oriented with a steeper downward angle. Both leaf surfaces of apple and pear retained a higher proportion of microcapsules than walnut leaves, and the bottom surface of apple leaves retained significantly more than pear leaves. Three spray adjuvants were evaluated as stickers for microcapsules. No difference was found in the number of microcapsules deposited on apple leaves among three stickers tested at rates from 0.06 to 0.25%. However, in a second test a latex sticker significantly increased the deposition of microcapsules on apple leaves compared with a polyvinyl polymer and a pine resin sticker at a rate of 0.06%. Significantly more microcapsules were retained on the bottom versus the top of apple leaves with all stickers. The latex and polyvinyl stickers significantly increased the retention of microcapsules versus the pine resin sticker and the control on apple leaves. In another test, the addition of 0.06% latex sticker did not increase the deposition of microcapsules on any of the three leaf types. However, the addition of the latex sticker significantly increased the retention of microcapsules on the top of apple and pear leaves and the bottom of apple leaves. The addition of a latex sticker did not affect the retention of microcapsules on walnut leaves.

**KEY WORDS** rain, stickers, walnut, apple, pear

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CODLING MOTH, *Cydia pomonella* (L.), is a key pest of pome fruit and walnuts throughout the major growing regions of the world (Barnes 1991). In the western United States, codling moth has been managed primarily with organophosphate insecticides for >40 yr (Croft 1983). However, the adoption of sex pheromones to achieve control of codling moth through mating disruption increased rapidly during the 1990s (Brunner et al. 2002) and has reduced the use of these broad-spectrum insecticides by up to 70% in some contiguous areas of fruit production (Calkins et al. 2000). Various hand-applied plastic dispensers have been the most widely used approach in sex pheromone-based mating disruption of codling moth in North America (Thomson et al. 2000). However, the adoption of hand-applied dispensers has been limited in walnut due to the difficulty of applying dispensers in the upper canopy of trees 15 m in height (Shorey and Gerber 1996). Concurrently, the use of microencapsulated sex pheromone formulations has been de-

veloped for a number of important lepidopteran pests, including codling moth (Moffitt 1978, Knight 2000). The ease of application, compatibility with most pesticides, and the increased flexibility in adjusting the rate and timing of applications are major factors favoring the use of sprayables versus the hand-applied dispensers (Hall and Marrs 1989).

Conversely, the major limitation affecting the adoption of sprayable pheromone formulations has often been their relatively short residual activity (Campion 1976). Stabilizing the conjugated diene, (*E,E*)-8,10-dodecadien-1-ol, codlemone, and maintaining a consistent release rate over time have been the major difficulties in developing an effective dispenser or formulation for management of codling moth (Brown et al. 1992, Millar 1995). Recent work with microcapsules has reported improved protection of codlemone from degradation by sunlight (Eng et al. 2003). Additional efforts to extend the activity of microcapsule formulations for codling moth could speed the adoption of this technology.

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Improving the rates of deposition and retention of microcapsules in the canopies of host crops may be another approach to improve the efficacy and extend the longevity of microencapsulated formulations for codling moth. Differences in spray technology affecting the number, size, and distribution of spray droplets will likely influence the deposition of microcapsules. Physical factors such as seasonal differences in leaf trichome density and wax among crops, cultivars, and leaf surfaces may be additional factors influencing the retention of microcapsules (Plourde et al. 1985). Dislodgement of microcapsules from leaf surfaces by water could be a major factor limiting the longevity of these aqueous formulations. Overhead irrigation is commonly used in orchards in the western United States and can be a major factor in removing pesticide residues (Westgard et al. 1974; Howell and Maitlen 1987). Other orchards also are periodically cooled with overhead watering to reduce fruit sunburn and enhance color during the warmest period of the summer (Williams 1993). In spite of the fact that rainfall accumulation is generally low in the major pome fruit-growing regions of the western United States, the occurrence of precipitation after spray applications has been linked to pest control failures (McMechan et al. 1972). The use of adjuvants such as spray stickers could enhance the adhesion of microcapsules to foliage. Herein, we examine the effects of simulated rainfall and the addition of stickers on the retention of microcapsules formulated with codlemone on apple, pear, and walnut foliage.

### Materials and Methods

**Laboratory Test Protocol.** Studies were conducted with an experimental microencapsulated product formulated with 15.68% codlemone and 0.10% fluorescent dye ("Dye-Lite" Tracer Products, Westbury, NY), by Suterra LLC (Bend, OR). A standard concentration of product (200  $\mu$ l per 200 ml of water) was used in all tests. This concentration deposited a consistent density of microcapsules, mean (SE) = 1.58 (0.03) per  $\text{cm}^2$ . The diameter of the microcapsules ranged in size from 50 to 150  $\mu$ m.

Foliage of fully mature apple 'Red Delicious', walnut 'Chandler', and seedling pear from greenhouse grown trees were used in these tests. The mean (SE) leaf areas for apple and pear leaves and walnut leaflets were 22.2 (1.1), 19.0 (0.6), and 22.8 (1.3)  $\text{cm}^2$ , respectively. All leaves were first rinsed in distilled water and allowed to air dry before treatment. Leaves were sprayed individually with a 0.5-ml solution using a 200-ml hand-pump aerosol polypropylene spray bottle (United States Plastic Corp., Lima, OH). Leaves were placed horizontally on a grid of pins embedded in paraffin, sprayed, and air dried for  $\geq 2$  h before fluorescent microcapsules were counted under UV illumination (Black-Ray Long Wave UV Lamp, Ultra-Violet Products, Inc., San Gabriel, CA). In tests evaluating the addition of stickers, leaves were exposed to a 30-min simulated sunlight exposure (1,350  $\text{KJ/m}^2$ ) to cure the stickers before testing (Suntest

CPS+, Atlas Material Testing Technology BV, Gelnhausen, Germany). After the initial count of microcapsules, groups of 5–10 leaves were clipped to a circular aluminum stand and placed into a research track sprayer (DeVries Manufacturing, Hollandale, MN). Clipped leaves were typically oriented at a downward angle of 0–30° in the leaf holder. The downward angle of the leaves placed in the aluminum holder was adjusted in one test by placing a wire under the leaf. The amount of simulated rainfall was adjusted by using a range of nozzles (Teejet 110° Series, Spraying Systems Co., Springfield, IL) and by adjusting the speed of the sprayer. Simulated rainfall was applied for 3 min unless otherwise noted. Leaves were allowed to dry and the number of microcapsules remaining on leaves was counted under the UV light.

**Effect of Simulated Rainfall.** Studies were conducted with apple leaves to evaluate the influence of rainfall intensity, rainfall duration, and leaf angle on the retention of microcapsules. Both the top and bottom surface of apple leaves were treated with microcapsules and rainfall was applied only to the top surface of leaves in each test. Apple leaves were exposed to simulated rainfall in the first test at rates of 1.2, 2.3, or 6.6  $\text{cm/h}$ . Rainfall was simulated for 3 min, and leaves were oriented at 20–30° in the holder. The effect of the duration of rainfall applications was evaluated at a rainfall rate of 6.6  $\text{cm/h}$ , and the number of microcapsules per leaf was recounted after 3, 15, and 45 min of rainfall. The influence of the leaf orientation was evaluated at a rainfall rate of 6.6  $\text{cm/h}$  for 3 min with apple leaves placed in the holder at either a downward angle of 0–10, 30–40, or 60–70°.

The effect of leaf type on the deposition and retention of microcapsules after a simulated rainfall event was evaluated for apple, pear, and walnut. Both the top and bottom of leaves were treated with microcapsules and each surface was placed dorsally in the holder at 0–10°. Rainfall was applied at a rate of 6.6  $\text{cm/h}$  for 3 min.

**Influence of Stickers.** Three agricultural adjuvants were evaluated as stickers for the microencapsulated codlemone formulation. StayPut (Helena Chemical Co., Collierville, TN) is a 1.0% (AI) polyvinyl polymer. Bond (Loveland Industries Inc., Greeley, CO) is a 45.0% (AI) latex spreader sticker with a proprietary surfactant deposition agent included. Nu-Film 17 (Miller Chemicals, Hanover, PA) is a 96.0% (AI) formulation derived from pine resin (di-1p-menthene). Tests were run with apple leaves to evaluate both the deposition and retention of microcapsules after a simulated rainfall applied at a rate of 6.6  $\text{cm/h}$  for 3 min. Each sticker was tested initially at three concentrations, 0.06, 0.12, and 0.25%. A second test evaluated the deposition and retention of microcapsules on apple leaves with each sticker at 0.06%. A final test evaluated the effect of adding 0.06% Bond on the deposition and retention of microcapsules to both leaf surfaces of all three crops.

**Data Analysis.** One-way and factorial analyses of variance (ANOVA) were used to evaluate differences in the deposition and retention of microcapsules on

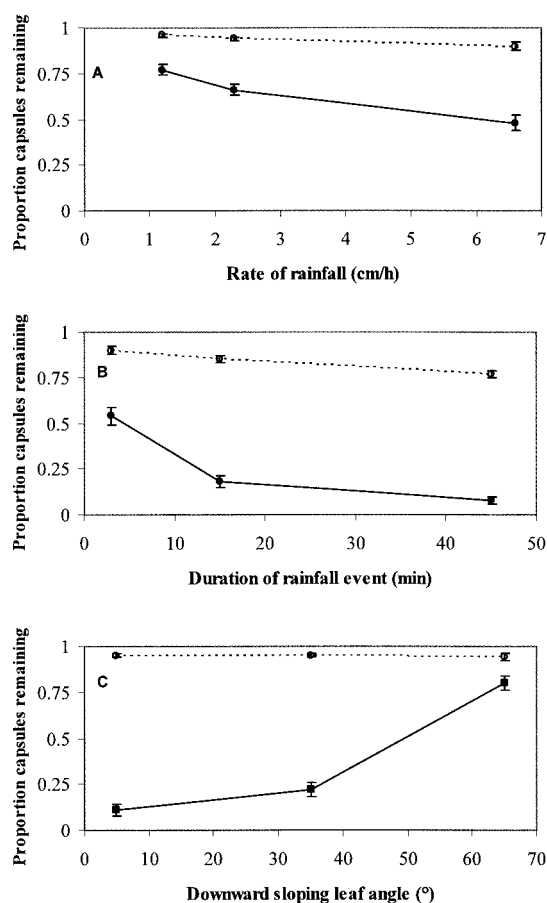


Fig. 1. Effects of intensity of simulated rainfall, (A) duration of the rainfall event, and (B) orientation of the leaf in the holder (C) on the retention of microcapsules formulated with codlemone on the top (●—●) and bottom (○- -○) surfaces of apple leaves.

both sides of apple, pear, and walnut leaves (Analytical Software 2000). Means were separated with least significant differences (LSDs) at  $P < 0.05$ . Five to 20 replicates were conducted with each test. Count and proportional data were transformed before analysis with square root ( $x + 0.01$ ) and arcsine (square root [ $x$ ]), respectively. Paired  $t$ -tests were used to compare the deposition and retention of microcapsules on both leaf surfaces of all three crops with and without the addition of Latex sticker. Linear regression was used to evaluate the retention of microcapsules as a function of the amount and duration of rainfall, as well as leaf angle in tests with apple leaves.

## Results

**Effect of Simulated Rainfall on Apple.** Both the intensity and duration of simulated rainfall events caused significant declines in the proportion of microcapsules retained on apple leaves (Fig. 1). The proportion of microcapsules remaining on apple

leaves declined with increasing rates of simulated rainfall (centimeters per hour) from both the top (slope [SE] =  $-0.052$  [0.008],  $r^2 = 0.42$ ,  $P < 0.001$ ) and bottom (slope [SE] =  $-0.010$  [0.003],  $r^2 = 0.14$ ,  $P < 0.05$ ) of leaves (Fig. 1A). A significant interaction between rate and leaf surface occurred in the factorial ANOVA ( $F = 12.09$ ;  $df = 2, 114$ ;  $P < 0.001$ ). A greater proportion of microcapsules were retained on the lower versus the upper surface of leaves at each rate,  $t$ -tests:  $df = 19$ ,  $P$  values  $< 0.001$ .

The proportion of microcapsules remaining on apple leaves declined with the duration of the simulated rainfall event from both the top (slope [SE] =  $-0.009$  [0.001],  $r^2 = 0.45$ ,  $P < 0.001$ ) and bottom (slope [SE] =  $-0.002$  [0.001],  $r^2 = 0.12$ ,  $P < 0.01$ ) of leaves (Fig. 1B). A significant interaction between rainfall duration and leaf surface occurred in the factorial ANOVA ( $F = 21.09$ ;  $df = 2, 114$ ;  $P$  values  $< 0.001$ ). Significantly greater numbers of microcapsules remained on the lower versus the upper surface of leaves for each exposure period,  $t$ -tests:  $df = 19$ ,  $P < 0.001$ .

The angle of the apple leaf in the simulated rainfall bioassay was an important factor affecting the retention of microcapsules (Fig. 1C). The proportion of microcapsules retained on the top surface of leaves increased significantly with increasing leaf slope (slope [SE] =  $0.012$  [0.001],  $r^2 = 0.72$ ,  $P < 0.001$ ). No significant relationship was found for the proportion of capsules remaining on the bottom of leaves with respect to leaf angle ( $P = 0.56$ ). A significant interaction was found in the factorial analysis between leaf angle and leaf surface ( $F = 93.54$ ;  $df = 2, 84$ ;  $P < 0.001$ ). Significantly more microcapsules were retained on the bottom versus the top of leaves at each leaf angle position tested,  $t$ -tests:  $df = 14$ ,  $P < 0.001$ .

**Deposition and Retention of Microcapsules among Crops.** There was no difference in the initial density of microcapsules deposited on either the top ( $F = 1.68$ ;  $df = 2, 42$ ;  $P = 0.20$ ) or bottom ( $F = 2.97$ ;  $df = 2, 42$ ;  $P = 0.06$ ) surfaces of apple, pear, or walnut leaves (Table 1). However, there were significant differences in the percentage of microcapsules removed from both the top ( $F = 3.88$ ;  $df = 2, 42$ ;  $P < 0.05$ ) and bottom ( $F = 19.82$ ;  $df = 2, 42$ ;  $P < 0.001$ ) of leaves occurred. A significantly higher percentage of microcapsules were lost from both surfaces of walnut leaves versus apple or pear leaves. In addition, significantly more microcapsules were lost from the bottom of pear versus the bottom of apple leaves (Table 1).

**Effect of Stickers on the Deposition and Retention of Microcapsules.** Neither sticker rate ( $F = 1.44$ ;  $df = 2, 125$ ;  $P = 0.24$ ) or sticker type ( $F = 1.22$ ;  $df = 2, 125$ ;  $P = 0.30$ ) influenced the density of microcapsules deposited on the top of apple leaves. The interaction of sticker type and rate was not significant in the first test ( $P = 0.52$ ). In a second test, there was a significant difference among stickers added at 0.06% and the use of no sticker in the density of microcapsules deposited on apple leaves ( $F = 4.44$ ;  $df = 3, 72$ ;  $P < 0.01$ ) (Table 2). Significantly more microcapsules were deposited with the addition of Latex sticker, whereas the other two stickers did not increase the deposition of micro-

Table 1. Initial deposition of microcapsules formulated with codlemone and a fluorescent dye on the top and bottom of apple, pear, and walnut leaves and their percentage of displacement after a simulated rainfall

Crop	Pheromone-treated surface of leaf <sup>a</sup>			
	Top		Bottom	
	Initial mean (SEM) no. of capsules	% loss after rainfall <sup>b</sup>	Initial mean (SEM) no. of capsules	% loss after rainfall <sup>b</sup>
Apple	24.4 (1.7)	84.8 (2.7)b	27.2 (1.7)	47.8 (5.7)c
Pear	26.8 (2.2)	84.3 (3.1)b	32.2 (2.7)	75.1 (3.4)b
Walnut	29.8 (2.4)	93.0 (2.1)a	36.3 (3.3)	86.1 (3.6)a

Column means followed by a different letter were significantly different at  $P < 0.05$ , Fisher's LSD.  
<sup>a</sup> The pheromone treated surfaces of all leaves were placed dorsally in the leaf holder before the simulated rainfall treatment at a downward 20–30° angle.  
<sup>b</sup> Simulated rainfall was applied for 3 min at a rate of 6.6 cm/h.

capsules versus the control. Significantly, more microcapsules were deposited on the bottom versus the top of apple leaves ( $F = 50.56$ ;  $df = 1, 72$ ;  $P < 0.001$ ). The retention of microcapsules on apple leaves after simulated rainfall was significantly different among treatments ( $F = 22.59$ ;  $1, 72$ ;  $P < 0.001$ ). The addition of either the latex or polyvinyl sticker retained significantly more microcapsules on leaves than the use of the pine resin sticker or the control (Table 2). Significantly more microcapsules were retained on the bottom than the top of apple leaves ( $F = 30.85$ ;  $1, 72$ ;  $P < 0.001$ ).

In a third test, the addition of 0.06% Latex sticker did not increase the density of microcapsules deposited on the top or bottom of apple, pear, or walnut leaves (Table 3). However, the use of Latex sticker did significantly increase the density of microcapsules retained after simulated rainfall on both the top and bottom of apple leaves and the top of pear leaves versus applications without the addition of the sticker. The addition of this sticker did not improve the deposition or retention of microcapsules in walnut (Table 3).

Discussion

The application of simulated rainfall to leaves treated with a sprayable formulation of codlemone had a significant negative impact on the retention of

microcapsules on apple, pear, and walnut leaves under laboratory conditions. Increasing either the intensity or duration of rainfall significantly increased the loss of microcapsule from apple foliage. However, the effect of natural rainfall or overhead irrigation under field conditions has not been reported. Rainfall during the summer in the arid tree-fruit and nut-growing areas in the western United States is uncommon. Over the past 10 yr, daily accumulations of rainfall during the summer have been low, i.e., 0.0–2.0 cm at our Yakima research farm. Further studies are needed to assess the retention of microcapsules after rainfall events that are more representative of field conditions. In contrast, orchards irrigated with overhead sprinklers are typically watered continuously for 12–24 h and could strongly impact the efficacy of sprayable sex pheromone formulations.

Leaf orientation was a key factor influencing the displacement of microcapsules in our tests. However, it is not clear whether this result was due to differences in the downward force of rain droplets, the degree of wetting of leaf surfaces, or the velocity of water running off of leaves for horizontal versus angled leaves. Microscopic examination of horizontal leaves after the simulated rainfall found that the remaining capsules tended to be larger than average ( $>100 \mu\text{m}$ ), and many capsules had been redeposited at the tip of the leaf. Leaf orientation on apple, pear, and walnut trees likely includes a full 360° range. However, this factor

Table 2. Deposition and retention of microcapsules formulated with codlemone on the top and bottom of apple leaves before and after a simulated rain event

Event	Sticker <sup>a</sup>	Mean (SE) no. capsules per leaf		
		Top of leaf	Bottom of leaf	Overall mean
Deposition of microcapsules before rain	None	23.6 (2.5)	38.3 (3.0)	31.0 (2.5)a
	Latex	32.3 (2.9)	46.7 (2.0)	39.5 (2.4)b
	Polyvinyl	24.0 (2.0)	39.3 (2.8)	31.7 (2.4)a
	Pine resin	30.2 (3.0)	38.1 (2.4)	34.2 (2.1)a
	Overall mean:	27.5 (1.4)a	40.6 (1.4)b	
Retention of microcapsules after rain	None	7.1 (0.8)	11.4 (1.3)	9.3 (0.9)a
	Latex	17.3 (1.0)	21.1 (1.8)	19.3 (1.1)b
	Polyvinyl	13.0 (1.3)	21.1 (1.6)	17.1 (1.4)b
	Pine resin	12.2 (1.1)	16.1 (1.2)	14.2 (0.9)a
	Overall mean:	12.4 (0.8)a	17.5 (1.0)b	

Column and row overall means followed by a different letter were significantly different at  $P < 0.05$ , Fisher's LSD.  
<sup>a</sup> All stickers were added at 0.06%.

Table 3. Mean (SE) no. of microcapsules deposited and retained on three leaf types with and without the addition of a latex sticker to a microencapsulated pheromone formulation before and after a 3-min period of simulated rain at 6.6 cm/h

Leaf type	Treatment	Mean (SE) no. capsules per leaf			
		Top of Leaf		Bottom of Leaf	
		Before	After	Before	After
Apple	Latex sticker	29.4 (1.5)	16.0 (1.5)b	32.8 (4.2)	15.0 (2.7)b
	No sticker	28.8 (2.2)	4.0 (0.7)a	27.3 (4.2)	8.5 (1.8)a
	<i>t</i> -tests: df = 38	<i>t</i> = -0.21 <i>P</i> = 0.84	<i>t</i> = 7.25 <i>P</i> < 0.0001	<i>t</i> = 0.93 <i>P</i> = 0.36	<i>t</i> = 2.32 <i>P</i> < 0.05
Pear	Latex sticker	21.5 (2.7)	10.4 (1.8)b	27.2 (3.6)	7.3 (1.1)
	No sticker	25.1 (2.5)	7.3 (1.1)a	28.4 (4.2)	6.3 (0.9)
	<i>t</i> -tests: df = 18	<i>t</i> = -0.97 <i>P</i> = 0.34	<i>t</i> = 2.07 <i>P</i> < 0.05	<i>t</i> = -0.21 <i>P</i> = 0.83	<i>t</i> = 1.03 <i>P</i> = 0.32
Walnut	Latex sticker	20.4 (1.9)	2.8 (0.9)	38.6 (3.3)	16.0 (3.9)
	No sticker	24.0 (2.5)	2.6 (0.2)	34.4 (4.7)	15.2 (3.0)
	<i>t</i> -tests: df = 8	<i>t</i> = -1.14 <i>P</i> = 0.29	<i>t</i> = 0.45 <i>P</i> = 0.67	<i>t</i> = 0.79 <i>P</i> = 0.48	<i>t</i> = -0.16 <i>P</i> = 0.88

Leaves were placed at 0–10°. One way analysis of variance (df = 3, 16) were conducted with transformed count data [ $\sqrt{x + 0.01}$ ]. Column means followed by different letters were significantly different at *P* < 0.05.

has not been examined with regard to significant differences among crops (compound walnut leaves versus simple apple and pear leaves) or cultivars, including the effects of rootstock, spur or nonspur leaves, and pruning practices (a variety of trellis and non-trellis systems).

Leaf surface was an important factor influencing the deposition and retention of microcapsules in these tests. Microcapsules deposited on the bottom of apple leaves were somewhat protected from rainfall with <10% loss during the 3-min bioassay (Fig. 1). In addition, differences in the physical characteristics of the top and bottom of apple and pear leaves may have increased the retention of microcapsules compared with walnut leaves. Qualitative differences in the top and bottom leaf surfaces of the three crops were observed under a dissecting microscope. The top surface of apple leaves seemed waxy and was covered with small groups of trichomes scattered throughout the leaf surface. The bottom surface of the apple leaves had a much higher density of trichomes covering the entire underside of the leaf. In contrast, neither the top nor bottom surfaces of the pear and walnut leaves had trichomes. Extreme differences in both the occurrence and density of trichomes and qualitative and quantitative differences in epicuticular waxes can occur among leaves due to differences in crop, cultivar, and age, as well as between leaves of a greenhouse-grown versus a tree under field conditions (Plourde et al. 1985, Jetter and Schaffer 2001). Significant differences in the deposition of microcapsules for the oriental fruit moth, *Grapholita molesta* (Busck), were found due to the age of an apple leaf (Waldstein and Gut 2003). However, the authors could not assess whether differences in pubescence or cuticular waxes, or both, accounted for the greater number of microcapsules deposited on young versus old leaves.

The addition of stickers in our tests often had a significant positive effect on both the initial deposition and retention of microcapsules on leaves. Microcapsules formulated with codlemone have some degree of natural adhesiveness due to the sticky characteristics

of the sex pheromone (Brown et al. 1992). Whether changes occur in the stickiness of microcapsules due to temporal changes in the emission characteristics of aging microcapsules was not investigated. The emission profile from microcapsules typically shows an initial high rate followed by a steep drop, and then a gradual decline (Hall and Marrs 1989). The fate of microcapsules deposited on leaves over an extended period in the field should be investigated, as well as a measure of their adhesion. The influences of wind on leaf movement and the deposition of dust on the dislodgement of microcapsules from foliage has not been investigated and could be significant, especially along the more exposed physical boundaries of tree and orchard canopies.

The application of microcapsules with an air blast sprayer distributes microcapsules fairly uniformly on leaves throughout the canopy (Knight and Larsen 2004), and likely on other plant parts, such as bark and fruit. Differences in the deposition and retention of microcapsules among plant parts may be significant. For example, the density of microcapsules deposited on 1-yr apple wood was greater than on leaves when tissues were dipped into a solution (Waldstein and Gut 2003). Whether this greater affinity for new wood was due to differences in surface texture or chemical composition between stems and leaves was unclear.

Sex pheromone-loaded microcapsules have been used to effectively manage lepidopteran pests of horticultural crops with either frequent application of low rates (Polavarapu et al. 2001) or less frequent applications of high rates of sex pheromone (Trimble et al. 2003). Determining which strategy is most effective will likely depend on the behavior of the pest, the emission characteristics of the microcapsule, and the deposition and retention of microcapsules within the canopy of the crop. It is not yet clear for codling moth whether one of these approaches is more effective in disrupting sexual communication. We hypothesize that any technical adjustments in spray delivery methods that will increase the deposition and retention of microcapsules in the canopy also will increase the



efficacy of this technique. Our study suggests that the addition of a sticker and the reduction in spray volume to avoid runoff may improve mating disruption of codling moth.

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